invariably introduce a premature stop codon that results in truncation of the distal cytoplasmic domain of the receptor and deranged signaling (see panel B). It is hypothesized that the loss of terminal differentiation signals from the distal cytoplasmic domain contributes to a hyperproliferative phenotype and leukemogenesis. In 1 published series, the incidence of cytoplasmic truncation mutations was 78% in individuals with SCN and MDS or AML vs 34% in individuals without signs of malignant transformation. In rare instances, somatic or de novo heterozygous mutations affecting the extracellular domain of the G-CSFR have been reported to cause sporadic SCN (see panel C). Such mutations are thought to act in a dominant negative fashion by inhibiting receptor trafficking to the cell surface and oligomerization in response to G-CSF. These variants of SCN are unresponsive to high doses of recombinant G-CSF.

Now Triot et al identify a new subtype of SCN, characterized by recessively inherited, loss-of-function mutations in CSF3R, in 2 unrelated families. The affected children in the first family harbored a homozygous missense mutation p.Arg308Cys, near the WSXWS motif (see panel D), that resulted in abnormal G-CSFR glycosylation, impaired trafficking of the receptor to the cell surface, and reduced downstream signaling. Paralogous disease-causing mutations have been reported in other type I cytokine receptors. The affected child in the second family carried compound heterozygous frameshift mutations that truncated the receptor in the extracellular domain (p.Gly316fsTer322 and p.Gly415fsTer432). None of the affected children in either kindred responded to treatment with recombinant G-CSF.

Despite reduced numbers of circulating neutrophils, all the patients had morphological evidence of full myeloid cell maturation in the bone marrow. This contrasts with SCN caused by ELANE or HAX1 mutations, wherein an arrest in myeloid maturation is pathognomonic. Like the patients reported by Triot et al, mice harboring homozygous loss-of-function mutations in either G-CSF or its receptor have reduced numbers of neutrophils in the peripheral blood and full myeloid maturation in the bone marrow. Altogether, these studies in humans and mice support the existence of G-CSFR-independent signaling pathways that control myelopoiesis. Remarkably, it has taken 2 decades of analyzing patient samples to confirm predictions made on the basis of these genetically engineered mouse models, underscoring the premise that validation is a dish oft served cold.

In 20% to 30% of SCN cases, a genetic cause has not yet been identified. In the coming years, the molecular basis for other rare subtypes of SCN may be elucidated and shed light on the G-CSFR-independent signaling pathways that regulate granulopoiesis. The generation of induced pluripotent stem cells (iPSCs) from patients harboring biallelic loss-of-function mutations in CSF3R, such as those described by Triot et al, would facilitate the study of G-CSFR-independent myelopoiesis. Eventually, advances in genome sequencing technology and iPSC differentiation models may obviate the need for mouse models and their validation.

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Comment on Chen et al, page 3818

Erythroid DAMPs drive inflammation in SCD

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In this issue of Blood, Chen et al show that heme is released during erythrocyte hemolysis in sickle cell disease, activating the innate immune response and triggering the release of neutrophil extracellular traps (NETs) to promote lung injury.

Sickle cell disease is characterized by intraerythrocytic hemoglobin S polymerization that leads to vasoocclusive events and chronic hemolytic anemia. Hemolysis, although traditionally considered simply a cause of anemia and gallstone formation, has been shown to cause endothelial dysfunction and chronic vascular injury via the release of cell free plasma hemoglobin and arginase 1, which collectively reduce nitric oxide (NO) bioavailability and enhance reactive oxygen species (ROS) formation. Oxidation of hemoglobin can result in the release of free heme into plasma, which in excess has recently been shown to activate Toll-like receptor 4 (TLR4) and promote vasoocclusion and acute lung injury.
Chen and colleagues show that, in addition to activating TLR4, heme promotes the release of NETs by increasing intracellular neutrophil ROS formation.\(^1\) NETs are decondensed chromatin, decorated with granular enzymes such as neutrophil elastase, that are released into the plasma by activated neutrophils.\(^6\) NETs are postulated to ensnare and kill pathogens with their high concentrations of granular enzymes. Chen and colleagues show that the production of NETs by neutrophils during tumor necrosis factor-\(\alpha\) exposure promotes acute lung injury and death in sickle cell mice, akin to the toxicity of NETs observed in mouse models of transfusion-related acute lung injury.\(^7\)

The increasing appreciation that intravascular hemolysis with release of hemoglobin and its oxidation products can drive sterile inflammation, via heme–TLR4 activation, suggests that erythrocyte-hemolysis products can be considered damage-associated molecular pattern molecules (DAMPs). In this regard, they are similar to mitochondrial and cellular DNA, uric acid, adenosine, HMGB1, and other cytoplasmic and nuclear proteins that when released outside of the cell after tissue injury, cellular necrosis, and other stresses activate innate immunity and cause systemic inflammation in the absence of infection. DAMPs bind the same group of pattern recognition receptors, such as TLRs that mediate innate immunity to pathogens. Crystals of uric acid, a metabolic byproduct of DNA, can bind to the nucleotide-binding oligomerization domain–like receptors to potentially activate the NALP3 inflammasome and increase IL-1\(\beta\) production.\(^8\) Active erythropoiesis in the setting of hemolysis may generate excess uric acid from extruded red cell nuclei to activate this pathway. It is likely that systemic inflammation, oxidative stress, and infection in patients with sickle cell disease enhance the sensitivity of the innate immune system to erythroid DAMP molecules, such as extracellular heme.

The erythrocyte contains abundant antioxidant enzyme systems, such as super oxide dismutase, catalase, and the peroxiredoxins, and it forms a diffusional barrier that limits NO catalysis. Release of hemoglobin from erythrocytes during hemolysis sets in motion a cascade of molecular events that damage vascular endothelium and activate innate immune responses. Upstream NO reactions with oxyhemoglobin potently scavenge NO and inhibit its signaling.\(^2\) Oxidation of ferrous hemoglobin to ferric and feryl hemoglobin generate hydroxyl and lipid peroxyl radicals.\(^3\) These reactions promote vascular and renal injury, culminating in pulmonary hypertension and chronic kidney disease as patients age.\(^5\)–\(^11\) Although haptoglobin and hemopexin limit the circulating levels of free hemoglobin and heme, the near saturation of these systems is evident among sickle cell disease patients in steady-state. These systems may become overwhelmed during the intensification of red cell hemolysis that often occurs during vasoocclusive painful crisis and acute chest syndrome.

The finding that hemolysis and heme play an important role in both TLR4 activation and NET formation in experimental models of vasoocclusion and acute chest syndrome opens the door to new therapeutic strategies to limit sterile inflammation in sickle cell disease patients. Chen et al highlight the therapeutic utility of DNase I treatment and neutrophil ROS scavenging with N-acetyl-cysteine. Upstream treatment with haptoglobin and hemopexin as well as downstream inhibition of TLR4 and the NALP3 inflammasome should be explored.

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